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Is the Y chromosome of *Drosophila* an evolved supernumerary chromosome?

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Summary

The Y chromosomes of most *Drosophila* species are necessary for male fertility but they are not involved in sex determination. They have many puzzling properties that resemble the effects caused by B chromosomes. Classical genetic and molecular studies reveal substantial affinities between Y and B chromosomes and suggest that the Y chromosomes of *Drosophila* are not degenerated homologues of the X chromosomes, but rather that their Y chromosomes evolved as specialized supernumeraries similar to classical B chromosomes.

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Introduction

Sex chromosomes are morphologically distinct chromosomes that seem to be correlated to the sex of the carrier[1]. Classical examples are the X and Y chromosomes found in mammals, Drosophilids and certain plants, and the W and Z chromosomes that are characteristic for many butterflies, reptiles and birds[2-4]. The presence of an exceptional chromosome, i.e. the Y or W, in only one of the two sexes suggests that the heteromorphic sex chromosomes control sexual differentiation. However, this is generally not the case in invertebrates. In those invertebrates that have been studied genetically, the assumption that the number of homomorphic (i.e. X and Z) or heteromorphic (i.e. Y and W) sex chromosomes is crucial for sexual differentiation could not be substantiated[5-8]. In addition, heteromorphic sex chromosomes are completely absent in many invertebrates that have haplodiploid sex determination[9]. Sex determination of *Drosophila* involves a multigenic system that measures the X:autosome balance; it is not based on a simple chromosomal mechanism[5]. Thus, at least the evolution of sex chromosomes in *Drosophila* seems to be an accidental consequence, rather than the cause of the establishment of a genetic sex determination.

Multiple origins of sex chromosomes, non-conservation of sex determination mechanisms

Sex determination appears to have evolved along a large variety of pathways. Among vertebrates and invertebrates a rainbow of sex chromosomes and sex-determining mechanisms are known[2,4,9]. The genes involved in sex determination are not conserved at the DNA sequence level, and detailed analysis of the gene hierarchies in *Drosophila* and *Caenorhabditis* revealed that the formal mechanisms are not conserved either[5]. In some instances, such as the house fly *Musca domestica*[6] and poecilid fish[10-12], different variants of sex determination systems are realized within one species.

Classical theories about the evolution of sex chromosomes assume that the heteromorphic chromosomes arose from their homologous chromosomes after recombinational isolation and a progressive inactivation by Muller's ratchet[13,14]. In some cases it is obvious that the X and Y or the W and Z chromosomes are derived from a homologous autosome pair[2,4]. In higher vertebrates, the homology of the pseudoautosomal region between X and Y and its autosomal origin is beyond doubt. In other species, however, the descent of the sex chromosomes is obscure and based on the genomic localization of genes, pseudogenes, and repetitive DNA sequences that share DNA sequence homologies with the genes and pseudogenes that are present on the sex chromosomes. In *Drosophila miranda* a neo-Y chromosome evolved and molecular and cytogenetic analyses[15,16] have provided us with a snapshot of the inactivation and degeneration of the autosomal part of this chromosome. Among the Drosophilids, however, the formation of neo-Y chromosomes seems to be a rare event.

For neither *D. melanogaster* nor *D. hydei* could an evolution of the Y from the X chromosome be substantiated[17-19]. Although the Y chromosome of *Drosophila* segregates regularly from the X during meiosis, it does not undergo crossing-over with the X chromosome. As in all the other X-Y pairs of different organisms, regular segregation of the sex chromosomes has been used as the main argument for an

ancestral homology of these chromosomes. In order to explain the morphological and functional differences it has been assumed that the Y chromosome subsequently underwent gradual genetic inactivation, deletions and heterochromatization due to recombinational isolation[3,4,13,20]. Here we will present an alternative hypothesis for the origin of sex chromosomes that takes into account recent molecular and cytological information obtained in *Drosophila*.

The Y chromosome of *Drosophila* is not a degenerated X chromosome

In Drosophilids, all available evidence argues against a common ancestry of the X and the Y chromosome.

(1) Whereas the X chromosomes of the Drosophilids are highly homologous (the variations being restricted to the heterochromatic parts), the Y chromosomes are extremely variable in size and shape. At least in the *repleta* group, the primitive situation seems to be represented by small, dot-like Y chromosomes and not, as one might assume, by large, X-sized chromosomes[21].

(2) Genetic studies could not reveal the presence of functional, unique genes on the Y chromosomes of *D. melanogaster* and *D. hydei* that possess alleles on the X chromosome[19].

(3) Although X and Y chromosomes of many Drosophilids share ribosomal genes, intensive molecular studies failed to provide evidence for substantial DNA sequence homologies between X and Y: all genetic, cytological and molecular evidence gathered so far argues for the presence of a few fertility factors on the Y chromosome that are conventional, protein-encoding genes in a sea of heterochromatin (Table 1; ref. 19). Furthermore, the Y-chromosomal fertility genes do not possess truncated or non-functional alleles on the X chromosome. The few Y-chromosomal open-reading frames appeared to be defective pseudogenes or constituents of transposable elements[19,22].

(4) Neither the repetitive DNA sequences on the Y chromosomes nor their genomic distribution have been conserved in evolution[19,23]. Also, the genomic distribution of transposable elements does not provide arguments in favour of a homology between X and Y chromosomes in *D. melanogaster*[24,25].

(5) There are no indications for an evolutionary conservation of the number and localization of the Y-chromosomal fertility genes that form giant lampbrush loops in both *D. melanogaster* and *D. hydei*[19]. Extended studies failed to reveal an evolutionary conservation of the transcribed repetitive DNA sequences. Moreover, the proteins that bind to the transcripts of these loops are not conserved in evolution. With respect to the dynein-encoding parts of the fertility gene kl-5 of *D. melanogaster*, it has been shown that the member of the β-heavy chain dynein family with the highest homology to the Y-chromosomal member occupies an autosomal location[26].

(6) The presence of fertility genes on the Y chromosome is not an absolute necessity: the males of a number of *Drosophila* species lack Y chromosomes, but are fertile (Table 2). In *D. affinis* even one or two Y chromosomes can be present that are not required for male fertility[27].

(7) The highly repetitive rDNA genes differ between X and Y chromosomes of *D. melanogaster* and *D. hydei* by the presence of ribosomal cistrons with intervening sequences (IVS) only on the X chromosome. Moreover, the Y chromosome of *D. simulans* is devoid of ribosomal genes, which in this species are restricted to the X chromosome[17].

In conclusion, the classical arguments for the evolution of a sex-restricted chromosome are not valid for the Y chromosomes of *Drosophila*. All available evidence shows that at least the Y chromosomes of *D. melanogaster* and *D. hydei* are virtually new constructs (c.f. ref. 18).

Heterochromatic effects of the Y chromosome of *Drosophila*

As already mentioned, the Y chromosome of *Drosophila* is not involved in sex determination or realization; in most but not all cases it controls male fertility[28]. Whereas mutations in these fertility genes identify distinct, unique functions in spermatogenesis, mutations of the non-coding repetitive DNA sequences can cause a number of puzzling, normally dose-dependent effects. For example, Y-chromosomal heterochromatin suppresses position-effect variegation[29], and extra Y chromosomes in *D. melanogaster* cause mottling of the eyes[30]. Certain combinations of Y chromosomes can be lethal[28], and extra Y-chromosomal material can modulate crossing over frequencies[31]. Additional Y-chromosomal heterochromatin retards spermatogenesis in *D. hydei*[28].

It is obvious that the Y chromosome is not genetically isolated from the rest of the genome[32]. The Y-chromosomal *ABO* sequences of *D. melanogaster* interact with the *abo* gene on chromosome 2[17], and in both *D. melanogaster* and *D. hydei* autosomal and X chromosomal genes have been identified that interact with the Y-chromosomal lampbrush loops[19,33]. Such interactions are characteristic for meiotic drive chromosomes[34,35].

Y chromosomes are similar to B chromosomes

All these Y-chromosomal effects are most probably caused by different families of repetitive DNA sequences that account for the bulk of the Y-chromosomal DNA. Very similar phenomena can be caused by B chromosomes. In more than 1000 plant and several hundred animal species, such chromosomes have been identified as supernumerary, non-essential chromosomes, and it has been postulated that about 10% of all living species carry such chromosomes[36,37]. Their incredible diversity makes a more generalized description impossible, but B chromosomes are frequently heterochromatic and polymorphic, and their numbers vary among the members of a population. In gen-

Table 1. *The Y chromosome of* Drosophila *at a glance*

Characteristic feature	*Drosophila* species		Techniques used
	D. melanogaster	*D. hydei*	
Phenotypic effects of the loss of the whole Y chromosome	*XO* viable, normal but immotile spermatozoa	*XO* viable; normal but immotile spermatozoa at 18°C; severe, early morphological effects at 26°C	Light microscopy; crosses, induced non-disjunction
Phenotypes of the Y chromosomal male fertility genes	*ms(Y)*s have no obvious phenotypical effects, besides immotility of spermatozoa	*ms(Y)*s have no obvious phenotypical effects, besides immotility of spermatozoa	Induction of *ms(Y)*s by EMS, X-rays, γ-rays, P-elements; screen for male sterility= no progeny; light microscopy
Conditional mutations	Temperature-sensitive (*ts*) mutations	Temperature-sensitive (*ts*) mutations	At 18°C fertile; *D.h.* sterile at 25°C, *D.m.* at 25-29°C
Number of Y chromosomal fertility genes	6 complementation groups: *kl-5*; *kl-4*; *kl-2*; *kl-1*; *ks-1*; *ks-2*	6+X complementation groups: *A, B, C, N, O, Q; M?, P?*	Complementation tests (test for male fertility) in males of the constitutions: X/Y/Y, T(X;Y)/Y; T(X;Y)/A.Y (segmental aneuploidy)
Genes that do not form lampbrush loops	3 genes do not form loops: *kl-2; kl-1; ks-2*	1+X genes do not form loops: *B, M?, P?*	Complementation tests; cytology and cytogenetics
Genes that form lampbrush loops	3 complementation groups correlate with 3 lampbrush loops (one gene-one loop): *kl-5 (A); kl-3 (B), ks-1 (C)*	5 complementation groups correlate with 5 lampbrush loops (one gene-one loop): *A (thread), C (pseudonucleolus), N (tubular ribbon), O (club), Q (noose)*	Cytogenetics, N-banding, Hoechst 33258-staining; the different alleles of one locus may delete, modify, or leave the loop intact; no intragenic complementation to male fertility; *ts* alleles never affect loop morphology†
Size of the loop-forming fertility genes//not loop-forming genes	±4 Mb//1.6-3 Mb	260 kb-1.5 Mb (Miller-spr.); *thread+pseudonucleolus*: ±4Mb; *tubular ribbon+club*: ±6 Mb//not known	Cytogenetics; banding techniques; Miller-spreading of RNA transcripts‡; pulse-field gel electrophoresis; fluorescence in situ hybridization (FISH)
Major transcribed satellite/ repetitive DNA sequences	*kl-5*: $(AAGAC)_n$ and $(AAGAG)_n$ *kl-3*: $(AATAT)_n$ *ks1*: $(AAGAC)_n$ and $(AAGAG)_n$	Thread: $Y_L I$: $(180\text{-}171\text{-}171\text{-}171bp)_n$; $Y_L II$: $(77bp)_n$; *rally*: $(200bp)_n$; pseudonucleolus: $Y_L III$: $(GACA)_n$; *rally*: $(200bp)_n$; tubular ribbon: *YDh22*: (73 or 55 or 57bp) basis repeat; *club*: *YDh18*: $(GATTGAT)_n$; *noose:ay1*: (400 bp)$_n$	Molecular cloning, DNA sequencing; Southern/northern blotting; in situ hybridization on metaphase chromosomes; for *D.h.* only: fluorescence in situ hybridization (FISH) on loop transcripts
Gene products/protein-coding	Dynein-coding (β-heavy chain) by *kl-5*	Dynein coding by A (*thread*)?	Polyacrylamide gel electrophoresis (PAGE); electron microscopy; molecular genetics

Information is taken from refs 17, 19 and 23.
†In the case of *ms(Y)Q4*^{ts} of *D. hydei* it could be shown that neither the morphology of the lampbrush loop *noose* nor the transcription of the major repetitive loop constituents is affected.
‡Reviewed in ref. 28. There is only one report on Miller-spreading of lampbrush loops of *D.m.*, c.f. ref. 19.
D.m., *D. melanogaster*; *D.h.*, *D. hydei*.

eral, they do not undergo crossing-over with the A chromosomes that constitute the indispensable part of the genome and, as a consequence of this genetic isolation, they become a playground of evolution.

Only rarely, B chromosomes carry protein-encoding genes with clearly defined functions. Exceptions are the pisatin detoxification genes on B chromosomes of the fungus *Nectria haematococca* that provide biocide resistance[38]. Notwithstanding, they can have manifold and rather diverse effects. In plants, for example, they can reduce or increase cell-cycle duration, germination, growth, flowering time, seed set, vigour and fertility[36]. In animals they can influence developmental time, size, fertility and other parameters[36]. More specifically, the B chromosome in *Scilla autumnalis* affects the expression of an esterase locus on one of the autosomes[39]. B chromosomes may have beneficial or detrimen-

Table 2. Drosophila *species with fertile X/O males*

D. affinis
D. annulimana
D. auraria
D. longala
D. orbospiracula
D. pictiventris
D. thoracis

After Ashburner, M., ref. 54.

Table 3. *Molecular composition of B chromosomes*[a] *(ribosomal DNA sequences excluded)*[b]

Species	B-chromosomal DNA sequences	B-specific/ B-associated[c]	Also present on A chromosome set of related species?	Methods[d]	References
Plants					
Secale cereale (rye)	1.1 kb tandem repeat on long arm	B-specific	*S. vavilovii*	GSH, ISH	37
	3.9 kb tandem repeat on long arm	B-specific	No	GSH, ISH	37
	120 bp tandem repeat at both telomeric ends	Telomeres and interstitial sites of all A chromosomes	*Triticum aestivum*	FISH	71, 72
	480 bp tandem repeat at one end	Telomeres of all A chromosomes	*S. vavilovii*, *S. iranicum*, *S. montanum*, *S. cereale*	GSH, FISH	72, 73
Zea mays (maize)	1.1 kb tandem repeat in centromeric region	B-specific[e]	Unknown	DSGL GSH, ISH DNA seq	74
Brachycome dichromosomatica (Compositae)	176 bp tandem repeat in centromeric region (±10% of B chromosome)	B-specific	*B. ciliaris* var. *languinosa*, *B. eriogona*, *B. segmentosa* *B. multifida*	F-PERT GSH DNA seq	37, 75
Crepis capillaris (Compositae)	No B-specific DNA sequences found	–	–	GISH F-PERT GE	37
Animals					
Glossina austeni (tse-tse fly)	95 bp tandem repeat at centromere and telomeres	Centromeres of X, Y, L1 (but not L2)	*G. morsitans morsitans* *G. pallidipes*	$CsCl_2$ GSH, ISH	37
Glossina morsitans morsitans	65±5 bp tandem repeat at centromere and telomere	X centromere, telomeres, Y	Unknown	$CsCl_2$ GSH, ISH	37
Nasonia vitripennis (wasp)	171 bp tandem repeat	B-specific	*N. longicorna*, *N. girault*	DSGL	44
	154-214 bp tandem repeat	B-specific	No	GSH	
	183 bp tandem repeat (together >30% of B)	B-specific	No	DNA seq	
	94 bp tandem repeat	B-associated	*N. longicorna*, *N. girault*, *Trichomalopsis dubius*		
Eyprepocnemis plorans (grasshopper)	180 bp tandem repeat	All centromeres	Unknown	FISH	37
Drosophila subsilvestris	216 bp tandem repeat	Dot chromosome, all centromeres	*D. ambigua*	GE, ISH DNA seq	52
Petauroides volans (great glider; marsupialia)	200-600 bp repetitive DNA sequences	All centromeres, but not on Y	Unknown	MC+PCR GSH, FISH	76

[a]This table is based on Table 1 of ref. 37.
[b]See ref. 53 for a list of species carrying B chromosomes with ribosomal DNA sequences.
[c]B-associated DNA sequences also occur on A chromosomes.
[d]Abbreviations: GSH, genomic Southern hybridization; ISH, *in situ* hybridization; FISH, fluorescence ISH; DSCL, differential screening of genomic libraries; DNA seq, DNA sequencing; F-PERT, phenol-emulsion reassociation technique in formamide; GE, agarose or polyacrylamide gel electrophoresis of restriction enzyme-digested genomic DNA; $CsCl_2$, preparative $CsCl_2$ density centrifugation; MC+PCR, microcloning followed by amplification by the polymerase chain reaction.
[e]In cloned B-chromosomal DNA fragments, the 1.1 kb B-specific tandem repeat occurs interspersed with B-associated DNA sequences.

tal effects, but it is very difficult, though not impossible, to analyze all of these effects experimentally (c.f. ref. 37). A feature of many B chromosomes is that they can drive meiotic segregation to promote their spread in the genome or a population[34,40]. Indeed, B chromosomes were the first genetic elements that were recognized as 'selfish'[41,42].

At the molecular level, B and Y chromosomes also exhibit many similarities (Tables 1 and 3). As the *Drosophila* Y chromosomes, they consist predominantly of repetitive DNA sequences. These families of repetitive DNA sequences can be specific for the particular B chromosome (B -specific), or they can occur in other genomic positions as well (B-associated). In close association with these repetitive DNA sequences, in a few cases, single copy sequences have been identified (e.g. *Nectria*[38]) or made plausible (e.g. *Poecilia*[43], *Nasonia*[44,45]). Moreover, both Y and B chromosomes often contain rDNA sequences. Thus, a comparison between Tables 1 and 3 reveals that, in their molecular composition, Y chromosomes of *Drosophila* do not differ from B chromosomes principally: they can be interpreted as specialized supernumeraries that often carry genes involved in the control of male fertility.

The origin and evolution of B chromosomes

The genesis of B chromosomes has been largely a subject of

speculation[36,37]. There is some suggestive evidence that they can arise *de novo*[43,46,47], but it is sensible to assume that many B chromosomes have arisen from A chromosomes by at least three different mechanisms. Hybridizations between species[48] or Robertsonian fusions[49,50] are believed to have contributed to the generation of most of the B chromosomes, but it is also likely that the non-disjunction of A chromosomes might have promoted the formation of supernumeraries[36]. Molecular approaches that might help to clarify the descent of the B chromosomes are now becoming feasible in a few species (Table 3). However, the results of these studies generally do not allow the identification of the parental A chromosome. The genomic distribution of B-associated repetitive DNA families might suggest a descent from certain A chromosomes[51,52], but this observation is not conclusive: the comparative analysis of Y-associated families of repetitive DNA sequences in the different Drosophilids has revealed that the genomic distribution of such families of repetitive DNA sequences is not indicative of the evolutionary relationships between the chromosomes[18,19,23].

Once a proto-B chromosome has been created by one or the other of these initial events, it is assumed that loss or inactivation of the original gene content starts. After reducing or abolishing crossing-over with the A chromosomes, Muller's ratchet can start operating[13,14,53] and, simultaneously, repetitive DNA sequences and transposable elements will become fixed on the evolving B chromosome. To a large extent, it is a matter of chance which kinds of repeats become integrated into the B chromosome, and because these sequences evolve rapidly, it is not surprising that B chromosomes can acquire very divergent properties.

A new hypothesis for the origin and evolution of Y chromosomes

We suggest three steps in the evolution of the Y chromosomes of *Drosophila*. The initial step is the generation of a supernumerary chromosome, comparable with the evolution of B chromosomes, as outlined above; the second the stable integration of such a supernumerary chromosome into the genome; and the third the acquisition of a sex determination function or a male fertility gene. These steps are discussed below.

(1) We propose that supernumerary chromosomes arise from the small dot-like autosomes by non-disjunction[52,54]. Among the Drosophilids, the generation of supernumeraries by species hybridizations are unlikely, just as the generation by Robertsonian fusions. The Y chromosomes of the 'primitive' *Drosophila* species of the *repleta* group (where no Robertsonian fusions have taken place) are small[21]. These Y chromosomes are comparable in size with the dot chromosomes. In *D. melanogaster*, and most likely in *D. hydei* as well, the dot chromosomes do not undergo crossing-over, neither in males nor in females[55]. In addition, in metaphase preparations, about one half of the dot chromosome of both species is heterochromatic[17]. In *D. melanogaster*, two families of satellite DNA sequences, i.e. (AATAT)n and (AAGAG)n, which are found in adjacent megabase-sized regions on the dot chromosome, are present on both arms of the Y chromosome in a similar configuration[18]. This is the first molecular evidence for a relationship between the Y and the dot chromosome of *D. melanogaster*.

Non-disjunctions of dot chromosomes can be as frequent as 1 in 3000, and supernumerary dots are easily tolerated[54]. These are favourable conditions for the evolution of both B and Y chromosomes, since Muller's ratchet can operate on the supernumeraries that are already recombinationally isolated[13,14,53]. Such a supernumerary dot has the potential to evolve as a B or a Y chromosome, depending on the genes and families of repetitive DNA sequences that become fixed in this chromosome.

(2) The second step in the evolution of Y chromosomes is the acquisition of regular segregation. There are a number of arguments for a 'drive' origin of the Y chromosome of *Drosophila*. The heterochromatic character of the Y chromosome in many species is considered a relic of past suppression of Y drive[35,56]. A driver ancestry has also been postulated for the *Ste/Su(Ste)* pair of *D. melanogaster*[57], where X- and Y-chromosomal *Ste* copies interact. Driving sex chromosomes are known from many animals and many Drosophilids[58]. Most concern X chromosomes; and driving Y chromosomes appear to be rare: in 1977 Lyttle[59] even experimentally confirmed the predicted dynamics[56] of artificial driving Y chromosomes using population cages with *D. melanogaster* cultures. Therefore, it is not surprising that suppressors of sex chromosome drive have been discovered in many natural populations, since driving Y chromosomes will rapidly disappear, either through extinction of carrier populations or because of strong selection for inactivation of drive[35,56]. Many studies have found drive suppressors of sex chromosomes in *Drosophila* populations[35]. Moreover, theoretical studies have shown that the conditions for stable sex chromosome polymorphisms other than neutral are very restrictive[60]. Thus, there is ample scope for the origin of a driving Y chromosome that becomes subsequently controlled by suppressors of drive on the autosomes or X chromosome in *Drosophila*.

It is very tempting to assume that a potential step in the evolution of regular segregation involved trivalent formation. Tri- or multivalent formation during meiosis favours the loss of one of the potential X chromosomes by non-disjunction, thereby generating gametes with a euploid constitution. Alternatively, the Y chromosome may have gained regular segregation as a suppressor of X drive. Such Y chromosomes are known from *D. mediopunctata*[32]. Indeed, the 'empty' Y chromosomes of some *Drosophila* species (e.g. *Drosophila affinis*) may only have evolved as supernumeraries to stabilize X chromosome segregation (see also refs 61,62). In addition, several B chromosomes have been described that associate with the non-homologous X chromosome followed by regular

segregation of the B and X chromosomes to opposite poles[36]. The presence of rDNA sequences on many Y chromosomes may be interpreted in this context. McKee *et al.* demonstrated that the acquisition of few (i.e. eight!) 240-bp repeats, which are present within a single rDNA cistron, are sufficient to guarantee a proper segregation between X and Y chromosomes in *D. melanogaster*[63]. The significance of the NORs (nucleolus organizer regions) on many B chromosomes has been discussed in a similar way[37,53].

(3) There are two scenarios for the origin of the sex differentiation or male fertility functions of the supernumeraries. Such sex-determining genes could have been localized on the ancestral autosomal segment, or they could have been acquired at a later stage of sex chromosome evolution. Sex determination genes are known to evolve rapidly[64-68], and transposing sex determination genes have been postulated for several organisms[67,68]. The Y chromosomes of those *Drosophila* species that are fertile as XO males (Table 2) might represent an early stage of Y-chromosomal evolution. These Y chromosomes carry neither sex-determining genes nor fertility factors. They are true supernumeraries with B-like effects on the fitness of their carriers[27]. The vast majority of the *Drosophila* Y chromosomes, however, caught or saved a few genes that are essential for male fertility[26], thereby guaranteeing their existence in the male sex[19]. The existence of several hundred such male fertility genes on the autosomes has been demonstrated by genetic screens in both *D. melanogaster* and *D. hydei*[28,33].

This scenario shows how a stable heterogametic constitution of sex chromosomes can evolve. However, the heterogametic sex might need mechanisms for a dosage compensation. Experimental evidence in *D. melanogaster* has shown that one gene (i.e. *Sex-lethal, Sxl*) controls both sex determination and dosage compensation by sex-specific splicing[5,69]. *Sxl* is reasonably conserved in the blowfly *Chrysomya rufifacies*[68], but there are no indications for an involvement of this gene in sex determination in *Chrysomya*. In contrast to *D. melanogaster*, there are no differences in the expression of this gene between the sexes, and there is no evidence for a sex-specific splicing of the transcript. A similar situation seems to exist in *Musca*[70]. Thus, even among flies, sex determination mechanisms are not conserved, despite a conservation of the key genes at the DNA and protein levels. This also suggests that the control of dosage compensation by upregulating transcription of whole chromosomes, if necessary at all, is 'easily' accomplished in evolution.

Conclusions

The incredible variability of sex chromosomes and sex determination mechanisms, and the absence of conservation of genes involved in sex determination at DNA levels or even formalistic levels, suggests that completely different mechanisms were operating during their evolution. While there is no doubt that the absence of exchange between homologous chromosomes can cause genetic degeneration[13,14] and a diverging evolution of an ancestral sex chromosome pair, the lack of homology between X and Y of the Drosophilids requires another explanation. The obvious parallels between Y chromosomes and B chromosomes suggest that supernumerary chromosome formation was important for Y chromosome evolution. Heterochromatin effects caused by the repetitive DNA sequences are known from many Y and B chromosomes, and Y chromosomes exist that do not carry any gene required for sex determination or male fertility. Sex-determining genes are likely to be transposable in several systems, and one can imagine that the transposition of a major sex-determining gene to a preexisting supernumerary chromosome can easily occur. Molecular analyses of the *Drosophila* Y chromosomes have identified several characteristics that can be considered as relics of a former driving nature. We expect to find more supportive evidence for the supernumerary origin of heterologous sex chromosomes when molecular data from other systems become available.

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